

PROJECT NUMBER: 6902
PROJECT TITLE: Biochemical Special Investigations
PROJECT LEADER: B. D. Davies
PERIOD COVERED: November, 1989

I. NICOTINE SPECIFIC MONOCLONAL ANTIBODY

- A. Objective: To obtain a monoclonal antibody (MCA) against nicotine (NIC-MCA).
- B. Results: It was determined that clone BE2 antibodies are of the IgM class. This completes BE2s characterization, a memo describing the complete characterization will be issued.

Screening was begun on media from several new clones. Three of ten clones possessed the required titer and levels of antigen specificity. One of these three, HA4, was further tested in the nicotine inhibition assay. Its 50% inhibition constant (IC50) was determined to be $3.98\text{E}-6$ M.

An additional batch of succinylated hydroxyethylnicotine-human serum albumin (SHN-HSA) was synthesized and characterized.

- C. Plans: Continue screening and characterizing positive clones. Extract tobacco with water at pH ~7 and 5% acetic acid, compare the efficiency of extraction by submitting both samples to ARD for nicotine analysis. Test the water extract in an ELISA. Send the SHN-HSA to Hazleton Laboratories for the production of polyclonal antibodies.
- D. Reference:

Crockett, E. Notebook No. 8863, p. 111.

II. ADDITIONAL APPROACHES TOWARD PUTRESCINE METHYLTRANSFERASE (PMT) ISOLATION

- A. Objective: Provide additional experimental approaches to assist in the effort to isolate PMT.
- B. Results: Samples of photolyzed and nonphotolyzed PMT were fractionated on Phastsystem IEF gels. In this preliminary study, the photolyzed sample appeared smeared and only faint banding patterns were visible. This was in contrast to the untreated sample which (like the control) gave distinct banding patterns.
- C. Plans: Conduct additional studies to determine the actual effects of photolysis of PMT material on its migration characteristics in IEF gels.
- D. Reference:

Crockett, E. Notebook No. 8863, p. 111.